وحـــدة الفيرولوجـــــى
معهد بحوث صحـــة الحيوان
الدقـى ـ القاهـــــــرة
رئيس الوحـدة : أ٠د / حسن السواح

التفاعل بين فيروسات الطاعون البقـري والاسهال الفيروســى والتهاب الجيوب الانفية المعدي فى المزارع النسيجيـة

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تم حقن مزارع نسيجية لخصية عجول بكل من الفيروسات الثلاثة على حدة وكذلك تبادليا بفيروسين معا أو بأحد الفيروسات الثلاثة ثم بعد الحقن بساعة وفى بعض الحالات بعد ٤٨ ساعة فى حقن نفس الانسجة تبادليا بأى من الفيروسين الاخرين ٠

أظهرت النتائج أن الانسجة التى حقنت بفيروس الطاعون أو الاسهال الفيروسى ثم بعد ساعة بفيروس الاسهال أو الطاعون وكذلك الانسجة التى حقنت بالفيروسين معا أدي الى تغيرات خلوية ترجع الى فيروس الطاعون البقري ٠

الانسجة التى حقنت بفيروس الطاعون والتهاب الجيوب الانفية المعدي معا والتى حقنت بفيروس الطاعون ثم بعد ساعة بفيروس التهاب الجيوب الانفية وكذلك فى الانسجة التى حقنت بفيروس الاسهال والالتهاب بالجيوب الانفية معا ظهر منها تكسر شديد بالخلايا ٠

الانسجة التى حقنت بفيروس الاسهال ثم بعد ساعة بفيروس التهاب الجيوب الانفية أو التى حقنت بفيروس الطاعون ثم بعد ٤٨ ساعة بفيروس التهاب الجيوب الانفية أظهرت بها تغيرات متأخرة وضعيفة ترجع الى فيروس التهاب الجيوب الانفية ٠ أو التى حقنت بفيروس الطاعون ثم بعد ٤٨ ساعة بفيروس الاسهال ٠

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INTERACTION BETWEEN RINDERPEST, BOVINE VIRAL DIARRHOEA AND INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN TISSUE CULTURE

(With One Table & Three Figs.)

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SUMMARY

In secondary calf testis cell cultures, infection with both RP and BVD viruses at the same time (mixed infection) resulted in cytopathic effect (CPE) which was mainly related to RP virus. The cytopathic effect related to IBR predominated after its use with either RP or BVD in mixed infection. No. blocking effect was observed for BVD virus in clutures infected with this virus followed after sometime by RP virus (superinfection). The virus of IBR was severely destructive, and the cultures superinfected with this virus followed either with RP virus or BVD virus did not surive long. However, the cytopathic effects in cultures infected firstly with BVD virus followed by IBR virus was slightly delayed. The results were explained on the basis of interferon production, and their importance for vaccine production, mainly RP vaccine, was discussed.

INTRODUCTION

Rinderpest virus (RP-virus), bovine viral diarrhoea virus (BVD-virus) and infectious bovine rhinotracheitis virus (IBR-virus) are three heterologous unrelated viruses (ANDREWES, et al. 1978). The cytopathic effect of each of these three viruses were separately investigated in cell culture of different systems. Calf testis cell culture was found to be susceptible for replication of BVD virus (GUTENKUNST and MALMQUIST, 1965). The testis of bovine embryo can maintain the growth and replication of IBR-virus (MADIN, et al. 1956; GRUNDER, et al. 1960; SCHIMMELPEFENNIG and LIESS, 1961).

In the present study, the mutual influence, in the form of interferrence of enhancement of infection as indicated by the cytopathic effects in tissue cultue, between these three viruses were studied in one cell system, namely secondary culture of calf testis. The cultures were infected either with one or another of the three viruses, with two viruses at the same time, or by superinfection in which there were a probable blocking (interferring) virus that was applied firstly, followed by a second superinfecting (challenge) virus.

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MATERIAL and METHODS

Tissue Culture:

Primary culture of calf testis were prepared by the conventional methods and the secondary culture was used in all studies. The cells were distributed into Roux bottles and incubated at 37°C until a confluent sheet was formed. Growth medium consisted of minimal essential medium (MEM) suplemented with 10% calf serum and 10% tryptose phosphate broth. The cells were then trypsinized and distributed in 6-mm diameter Petri dishes containing coverslips. The Petri dishes were incubated at 37°C in 5% CO_2 atmosphere. The maintainance medium consisted of MEM with 2% calf serum.

Viruses:

Three viruses, namely, RP-virus, BVD-virus and IBR-virus were used for infection. RP-virus was a vaccinal strain which was propagated on bovine kidney cells and manufactured by Animal Vaccine Production and Research Institute, Abbasia, Cairo, Egypt. This strain was passaged several times in Dokki Virological Lab. then passed twice on secondary calf testis cells before use.

The "Singer strain" of BVD-virus used in this study was obtained from the Faculty of Vet. Med., Cairo University, while the "AG strain" of IBR-virus was kindly supplied by Dr. BARTHA, ADORJAN, HUNGARY.

The three viruses were passed twice on secondary calf testis cells and titrated by the microtechnique on the same culture. The titre was determined according to REED and MUNCH (1938) and reached 10^2, $10^{4.5}$ and $10^{5.5}$/50 ul for RP, BVD and IBR viruses respectively.

Inoculation:

Petri dishes containing monolayers on coverslips were inoculated with 0.1 ml of RP-virus or the dilution 1:100 of BVD and IBR-virus. The cultures were infected by use of either a single virus, two viruses simultaneosly (mixed infection) or by superinfection. The scheme of work is shown in table 1. When a single or two viruses were used, the inoculum was adsorbed at 37°C for one hour, then discarded and replaced by the maintainance medium before incubation. In superinfection the first virus was adsorbed for one or more hours, the inoculum was discarded and followed by adsorption of the other virus for another one hour. Two Petri dishes were used for each inoculation. Control non-infected cultures for each treatment were always parallely incubated.

Staining Procedure:

After incubation, the cultures were fixed without washing in a mixture of ether-alcohol (1:1) for 20 minutes and then kept in 70% alcohol. The cultures were stained with Harris haematoxylin and eosin and mounted in canada balsam.

RESULTS

In cultures infected with RP-virus only, characteristic cytopathic changes were seen after 24 hours. In these cultures, many cells showed intensely eosinophilic intracytoplasmic inclusion bodies which were surrounded by unstained halo. The inclusion bodies at these stage appeared rounded, oval, or elliptical and has a regular smooth outline (Fig. 1). They were located mostly apart from the nucleus and did not cause much distorsion of the cell. In cultures examined

after 48 hours and 96 hours of infection, intracytoplasmic inclusions occurred more frequently in the cells. These inclusions were slightly larger in size, maintained their uniform and regular shape and may be multiple. At this stage, also, multinucleated giant cells in a very few numbers were observed (Fig. 2). The giant cells usually had a limited number of nuclei, cell vaculation or rounding were rarely observed and intranuclear inclusion bodies were not seen.

Infection of the cells with BVD-virus only, resulted in a slight changes. After 24 hours of infection, a few number of cells undrgo swelling and showed cytoplasmolysis. The changes remained also mild in cultures examined after 48 and 96 hours of infection, these changes consisted mainly of rounding with cytoplasmic processes.

In cell cultures infected with IBR-virus only, severe cytopathic effects occurred at all stages. After 24 hours, there were many empty spaces in the coverslips due to detachement of dead cells from the glass surface. The remaining cells were clumped together, shrinked and were rounded (Fig. 3). The cytoplasm stained intensely with eosin and in some cells it contained small irregular eosinophilic inclusion. Karyolysis was a common picture in the infected cells, and the nuclear membrane became folded giving the nucleus a lobulated appearance. No inclusion bodies were seen inside the nuclei. More cells were affected and appeared necrotic after 48 hours.

At this stage, the cultures were severely affected with the occurrence of shrunkn cells, cells debris and granular eosinophilic materials widely separated by empty spaces.

Simultaneous infection of the cultures with both RP and BVD viruses revealed the formation of inclusion bodies which, however, were irregular, faintly stained and less frequent. Cultures infected with both RP and IBR viruses were severely affected with rounding and shrinkage of the cells. Tiny intracytoplasmic irregular inclusions were infrequently demonstrated. A similar picture was found also in cultures infected with both IBR and BVD viruses.

In cultures in which RP virus was left to be adsorbed for one hour and inoculated further with BVD virus, i.e. superinfection, the cytopathic effects were mainly that related to RP virus. Eosinophilic intracytoplasmic inclusions were demonstrated in these cultures both after 24 and 48 hours. However, these inclusion bodies were more or less irregular in form, and appeared lightly stained after 48 hours of infection.

In cell cultures adsorbed with RP virus, using IBR as the challenge virus led to severe destruction. Most cells undergo shrinkage and rounding, and the degenerating cells were widely separated by empty spaces. Intracytoplasmic inclusion bodies were not found in these cultures.

Using BVD virus as a probable blocking virus, which was left to be adsorbed to the cells for one hour before inoculation of RP virus, revealed the occurrence of cytopathic changes which were quite similar in degree and nature to that seen in cultures infected with both viruses simultaneosly, and cultures infected with RP virus followed by BVD virus. When IBR virus was used for infection of these cells instead of RP virus, the cytopathic changes related to IBR virus eppeared to be delayed. Shrinkage, rounding and destruction of the cells in these cultures were mild after 24 hours while the changes after 48 hours resembled in degree the cultures infected with IBR virus alone for 24 hours.

In cultures inoculated with IBR virus as a probable blocking agent, the cytopathic effect in the form of cell destruction and fragmentation related to this virus were very severe and overshadowed the changes that might occur in cultures secondly infected with either RP or IBR virus.

Superinfected cultures with RP virus for 48 hours followed by IBR virus for 96 hours showed delayed effect in which cytopathic changes related to IBR virus were less severe than in cultures infected with this virus only. Rounding and fragmentation occurred but to a less extent. Intracytoplasmic inclusion bodies were frequently seen in the cells. When BVD virus instead of IBR virus was used in these RP-infected cultures, a few number of cells remained attached to the glass surface of the coverslips. Degenerating cells were clumped together. Three inclusion bodies were very rarely seen in less severely affected cells but these were not demonstrated in necrotic cells.

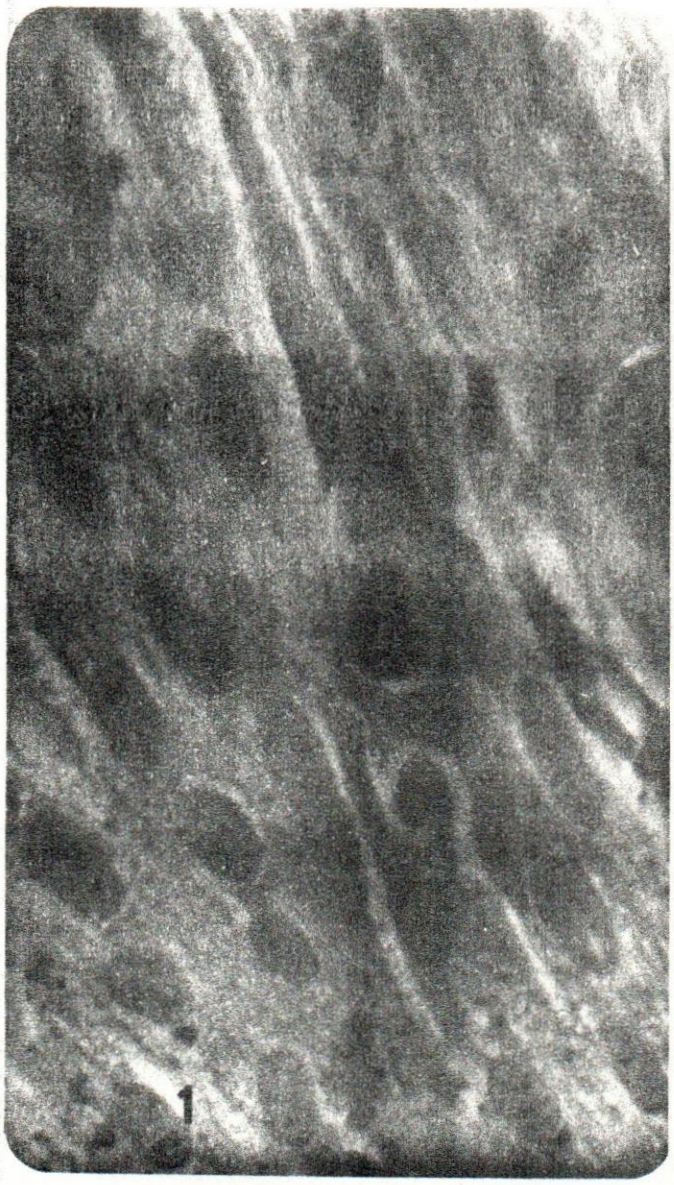
DISCUSSION

Study of the interaction between viruses may give important informations with respect to cellular machanisms and virus replication. The virus of RP was classified by WATERSEN (1962) as a myxovirus on the basis of its structural and functional features. On the other hand, the virus of BVD was reported by MERCHANT and PACKER (1969) to posses helical symmetry resembling the myxovirus. PLOWRIGHT (1964) suggested that culture adapted RP-virus matured and accumulated at the cell surface. This was confirmed by PROVEST, et al. (1965), who demonstrated that the multiplication of RP virus was restricted to the cytoplasm of infected cells and that it did not involve the mitochondria. They also showed that the virus was released by buding of the cellular surface. In general, as reported by MERCHANT and PACKER (1969) the site of synthesis of viruses of the myxovirus group was found to be the cytoplasm and depends on the cell membrane for their maturation; this may apply to BVD virus.

In the cultures superinfected with RP and BVD viruses, the cytopathic changes may led to the suggestion that, although the two viruses may not interfere with each others for attachement to the cell surface receptores, BVD virus may slightly compete with RP virus at the site of replication on the cytoplasm. This was manifested microscopically by the formation of irregular and faintly stained intracytoplasmic inclusions. Accordingly, it can be presumbed that RP-virus yield will be decreased. This assumbtion is of importance in cases of production of RP vaccine when the primary cultures, usually used in this process, be contaminated with a latent BVD virus.

As shown, in the present work, the cytopathic effect induced in cell cultures inoculated with BVD virus alone were very mild. It was difficult therefore, to make a conclusion whether or not that this virus be blocked when inoculated following infection of the culture with RP virus.

MERCHANT and PACKER (1969) reported that the morphology and structure of IBR virus is typical of the herpes virus group. Replication of the virus of this group begins with the formation of the nucleocapsid in the nucleus of the host cell. In the present study, RP and BVD viruses seen not to interfere either with penetration or replication of IBR virus in the infected cells. This appears to be due to differences in cell receptors and sites of replication. As has been reported by DEGUID, et al. (1978), interference of one virus with the replication of another may be mediated by a protein called interferon which is produced by the cell after infection of the first virus. It seems that, interferon itself is not the virus inhibitor but causes the cell to manufacture the protein which has a direct effect on virus multiplication. The protein called translation inhibiting protein, is thought to prevent translation of viral RNA.



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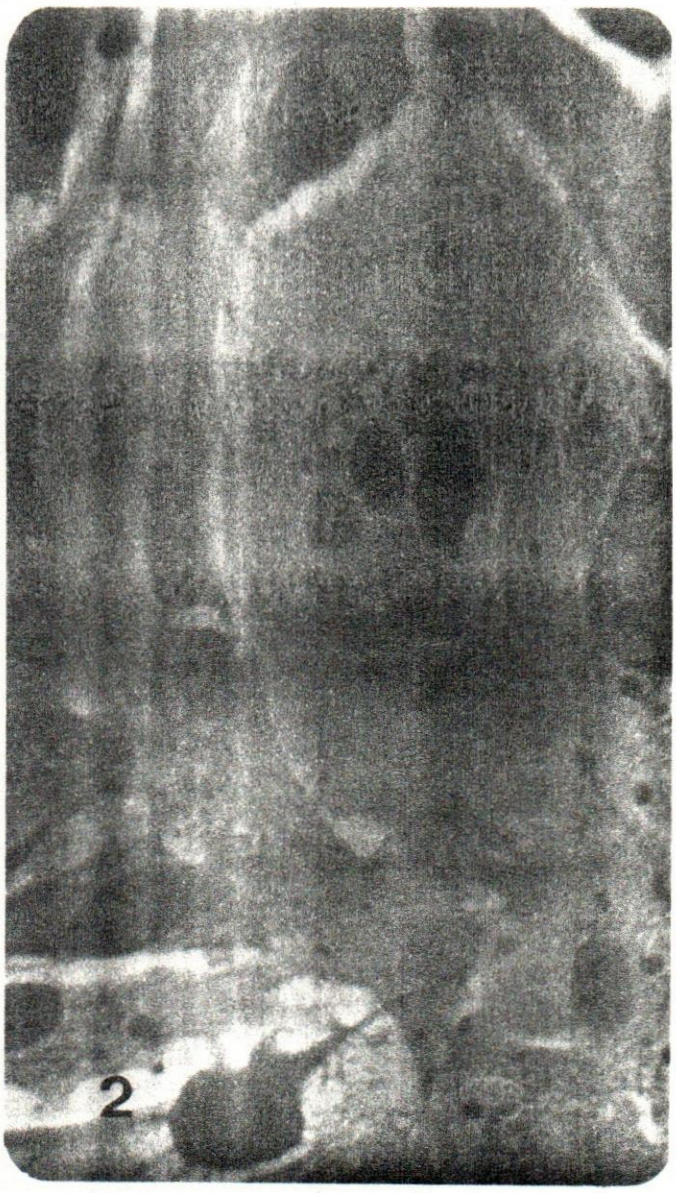

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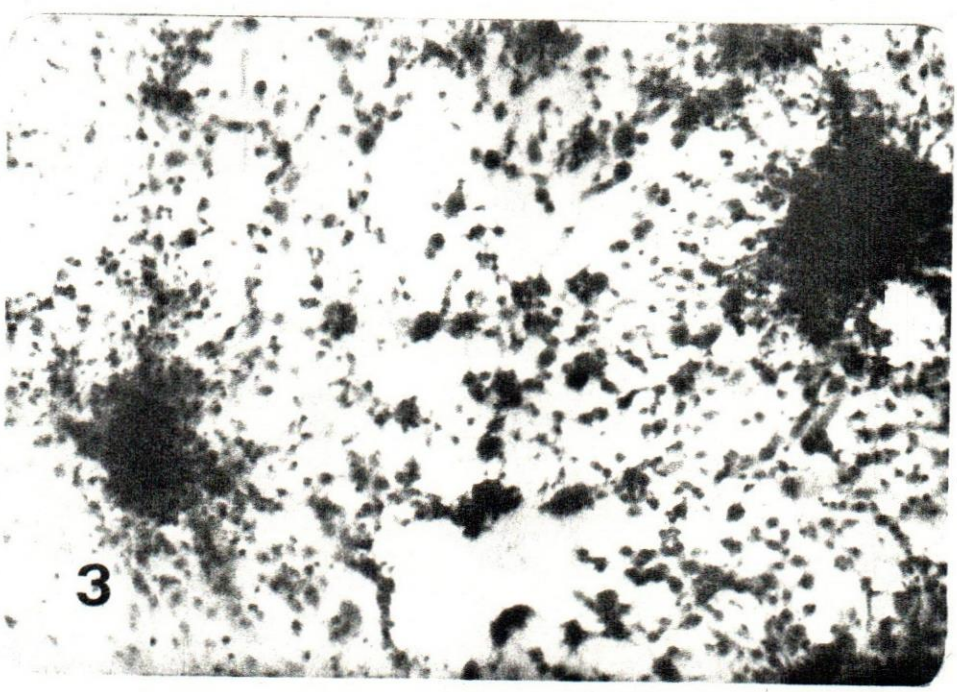

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